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14. ABSTRACT

The overall goal of this project is to determine the underlying mechanisms for the neurological symptoms associated with Gulf War Illness. The central hypothesis is that subthreshold exposures to organophosphates-OPs (defined as exposures not associated with acute signs of toxicity) may have adversely affected axonal transport and/or myelin integrity in affected individuals. We are studying two OPs, a representative insecticide that was used in the first gulf war, chlorpyrifos (CPF), and a representative, nerve agent, diisopropylfluorophosphate (DFP) in rats. The first year of this proposal has been dedicated to Specific Aim #1: which has been designed to evaluate OP effects on axonal transport in the living rat brain using manganese-enhanced magnetic resonance imaging (MEMRI) of the optic nerve axonal projections from the retina to the superior colliculus. The following procedures have been conducted to date (N=4-6): 1) baseline MRI scans; 2) daily injections of vehicle or chlorpyrifos (3.0-18.0 mg/kg) x 14 days; 2) a second MRI scan on the day following the last drug injection; 3) a third scan after a 4 week (OP-free) washout period. For each animal, a separate 6 hour and 24 hour scan was performed after Mg2+ eye injection. The data analyzed to date appear to support our hypothesis that repeated OP exposure leads to persistent impairments in axonal transport in the brain of living animals.

15. SUBJECT TERMS
Organophosphate, Myelin, Axonal Transport, Magnetic Resonance Imaging, Gulf War Illness

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INTRODUCTION

The overall goal of this project is to determine the underlying mechanisms for the neurological symptoms (particularly cognitive deficits) that have been associated with Gulf War Illness. The central hypothesis we are testing is that subthreshold exposures to organophosphates (defined as exposures not associated with acute signs of toxicity) from insecticides or nerve agents may have adversely affected axonal transport and/or myelin integrity in affected individuals. We are studying two OPs, a representative insecticide that was used in the first gulf war, chlorpyrifos (CPF), and a representative, nerve agent, diisopropylfluorophosphate (DFP) in rats. The first year of this proposal has been dedicated to Specific Aim #1: which has been designed to evaluate OP effects on axonal transport in the living rat brain using manganese-enhanced magnetic resonance imaging (MEMRI) of the optic nerve axonal projections from the retina to the superior colliculus.

BODY

The first ½ of specific Aim 1 is nearing completion (chlorpyrifos exposure-MEMRI studies). To date we have accomplished the following:

1. Baseline MRI scans (after Mg^{2+} eye injection) have been performed on each test subject.
2. Subjects (N=4-6) have received daily subcutaneous injections of chlorpyrifos or vehicle (at the following doses) x 14 days:
    - Vehicle
    - CPF 3.0 mg/kg
    - CPF 10.0 mg/kg
    - CPF 18.0 mg/kg
3. A second MRI scan was performed on the day following the last drug injection (after another Mg^{2+} eye injection) in each animal.
4. A third scan was performed after a 4 week (OP-free) washout period (after the third and final Mg^{2+} eye injection) in each animal.
5. For each animal a separate 6 hour and 24 hour scan was performed after Mg^{2+} eye injection.

It should be noted that due to the throughput limitations of the Core Imaging Facility for Small Animals (CIFSA), we are only able to scan a maximum of 4 animals (once) per day (each scan takes from 1-1.5 hours). A few animals have died under anesthesia, which required a full repeat of the dosing and imaging procedures. The limitation of the number of animals imaged each day requires staggering of the cohorts and very tight logistics.

Data Analysis and Quantification

As images are acquired, they are being analyzed as follows:

To quantify Mn^{2+} enhancement, manually drawn regions of interest (ROI) are placed in 2D slices in various selected areas (eye ball, optic nerve, lateral geniculate superior colliculus) along the Mn^{2+} enhanced and contralateral non-enhanced areas. In addition to quantifying the *magnitude* of Mn^{2+} enhancement within manually defined regions of interest as a qualitative measure of transport rate, spatial characterization of enhancement along the optic nerve is performed on a voxel-by-voxel basis after initial injection. In this manner, the distance to the edge of Mn^{2+} enhancement in the optic nerve can be converted to a numeric rate. The enhancement ratio within the ROI is calculated by dividing the enhanced ROI intensity by the contralateral unenhanced ROI intensity.

Thus to date, approximately 20 (of the 24) total rats to be imaged for the chlorpyrifos MEMRI studies have been analyzed as follows:

At 6 and 24 hours after Mg^{2+} eye injection at each time point (i.e., baseline, washout day 1 and washout day 30) the approximate number of image slices have been analyzed per individual test subject are:

Eyeball = 60 slices
Optic nerve = 12-18 slices
Lateral Geniculate = 12-18 slices
Superior Colliculus = 15-25 slices

In Fig 1 (see Supporting Data section below) we have presented a preliminary figure where it appears that our hypothesis is correct (that OP exposure leads to persistent impairments in axonal transport). For the data included in this figure, we have analyzed and compared the superior colliculus in 4 animals administered the highest dose of chlorpyrifos (18.0 mg/kg) and 4 animals administered vehicle (at the 24 hr time point after the Mg^{2+} eye injection). It should be noted that when we finish the N=6 for each group for this portion of Aim 1 we will perform a full statistical analysis for all parameters resulting from MRI analyses (baseline, at the end of dosing, and after 30-day OP-washout). Analysis of variance (with repeated measures) will be used to assess the effects of OP dose, time point, and dose x time point interactions across all areas (eye ball, optic nerve, lateral geniculate, superior colliculus).

While we are a bit behind the schedule originally proposed, we factored in six months at the end of the 3 year project for data analysis, paper submissions, etc. While it is now clear that the imaging portion of this project will most likely take the entire 3 years of the project, we will be able to overlap the imaging and histology portions of the study, since the histology experiments are not limited by the capacity of the Core Imaging Facility for Small Animals (CIFSA).

KEY RESEARCH ACCOMPLISHMENTS:

- The first ½ of specific Aim 1 is nearing completion (chlorpyrifos MEMRI studies).
- A preliminary analysis of the data appears to support our hypothesis that repeated exposure to chlorpyrifos leads to persistent deficits in axonal transport.

REPORTABLE OUTCOMES:

- No manuscripts, abstracts, or presentations have been made to date. It is anticipated that the first manuscript from this work will be submitted around late Spring 2014.

- We have submitted a competitive renewal application to NIEHS and have used the preliminary data shown in Fig 1 from the DOD project. In the NIEHS application, we have proposed to conduct MEMRI of the septo-hippocampal pathways for studying axonal transport specifically in memory related pathways in the brain. We have also proposed in vitro trafficking techniques for studying axonal transport. While there is conceptual overlap between these two proposals, there is no direct overlap of experiments and it expected that each project should augment the other.

CONCLUSION

It is a bit early in this project to make any firm conclusions, although the data collected to date appear to support our hypothesis that repeated OP exposure leads to persistent impairments in axonal transport in the brain of living animals. We anticipate that there will be adjustments to our experimental protocols in order to finish all of the proposed experiments by the end of the 3 year project. We now expect that the imaging portion of this work will take all 3 years of the project, but with proper management and overlap of the histology experiments we should be able to complete all experiments by the end of the grant period.

REFERENCES

None

APPENDICES

None

SUPPORTING DATA

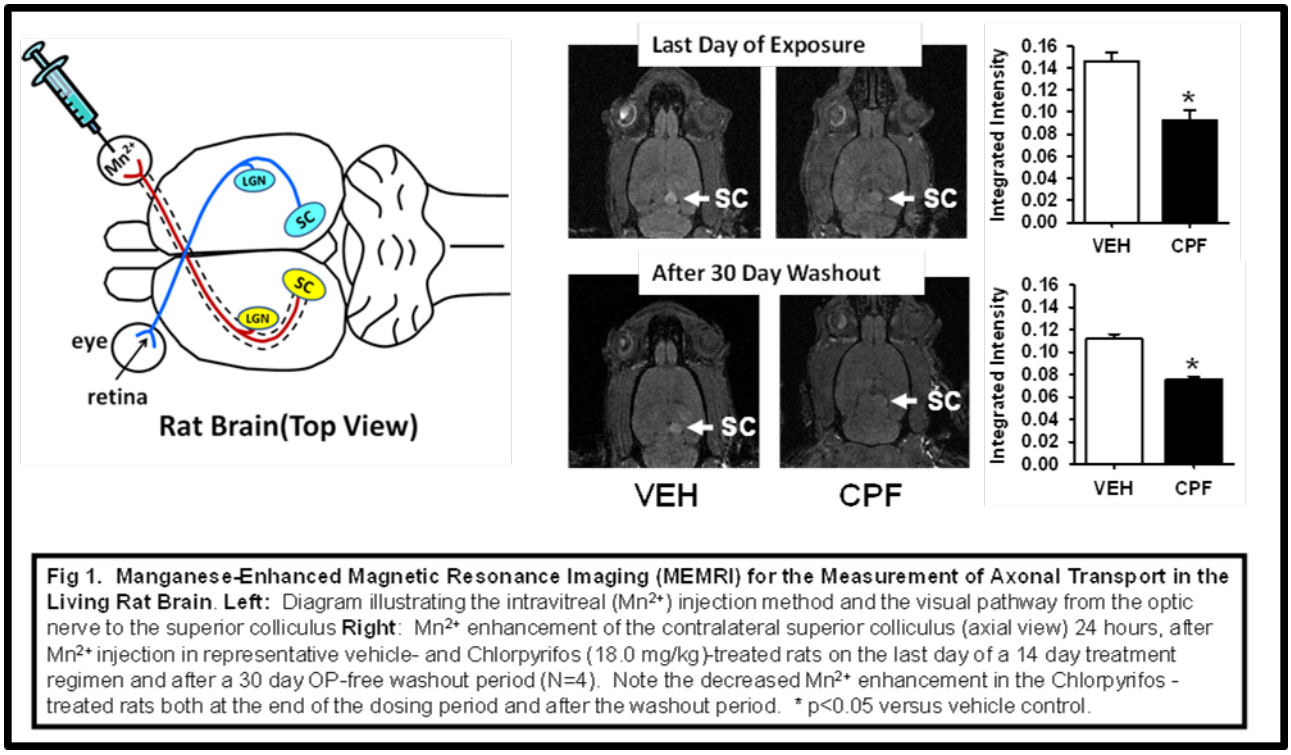

Fig 1. Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) for the Measurement of Axonal Transport in the Living Rat Brain. Left: Diagram illustrating the intravitreal (Mn^{2+}) injection method and the visual pathway from the optic nerve to the superior colliculus **Right**: Mn^{2+} enhancement of the contralateral superior colliculus (axial view) 24 hours, after Mn^{2+} injection in representative vehicle- and Chlorpyrifos (18.0 mg/kg)-treated rats on the last day of a 14 day treatment regimen and after a 30 day OP-free washout period (N=4). Note the decreased Mn^{2+} enhancement in the Chlorpyrifos - treated rats both at the end of the dosing period and after the washout period. * $p<0.05$ versus vehicle control.